MORPHOLOGICAL VARIATION IN HEMIPHRACTUS FASCIATUS (ANURA: HYLIDAE: HEMIPHRACTINAE)

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MORPHOLOGICAL VARIATION IN HEMIPHRACTUS FASCIATUS (ANURA: HYLIDAE: HEMIPHRACTINAE)

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SUMMARY

We describe morphological variation within the species *Hemiphractus fasciatus,* with special focus on populations in Panama, and determined the two allopatric populations are morphologically distinct, suggesting that there are at least two species involved in this system... The distinct groups are from eastern and western Panama, with the Panama Canal as the divider. Recent genetic analysis suggested there are three genetically distinct groups within *H. fasciatus.* The western specimens can be distinguished by cranial morphology, the texture of the skin under the throat.

CHAPTER 1

INTRODUCTION

The genus *Hemiphractus* was described by Wagler (1828) and several aspects of its taxonomy have been debated for over a century. *Hemiphractus* historically have ranged from Panama to northwestern Ecuador (Trueb, 1974), however their numbers are currently in rapid decline in some areas (Coloma et al., 2008). *Hemiphractus* are characterized as egg-brooding frogs with females carrying eggs and then young on the back (Duellman, Maxson, & Jesiolowski, 1988). *Hemiphractus* species have casqued skulls, with at least some co-ossification of the overlying skin, and with distinctive postorbital horns (Trueb, 1974).

Peters (1862) described *Hemiphractus fasciatus*, with the holotype from Pastaza Valley, Ecuador. Jiménez de la Espada (1871) described a new genus, *Cerathyla,* to accommodate the more "hylid like" of these horned frogs, in contrast to the "toad like" species he referred to *Hemiphractus*. In 1882 Boulenger described the new species *C. fasciata.* Then in 1917 Stejneger described *C. panamensis,* a species that was differentiated from *H. fasciatus,* based on type material collected near Santa Isabel, Provincia Colón, Panamá. *Cerathyla panamensis* was placed in the synonymy of *H. panamensis* by Noble (1926). Trueb (1974) placed *H. panamensis* in the synonymy of *H. fasciatus.* Although the holotypes were from Panama and Ecuador, respectively, she referred them to the same species.

Sheil et al. (2001) provided a phylogenetic tree for *Hemiphractus,* based on evidence from morphology (Sheil, Mendelson III, & da Silva, 2001), in which *H. fasciatus* was recovered as the sister species to *H. scutatus.* Recent genetic and morphological analyses recovered the same species pair, and suggested there are perhaps three different species currently classified under our focal species, *H. fasciatus* (Castroviejo-Fisher et al., 2015). The distribution of *H. fasciatus* sensu lato ranges from central and eastern cordilleras of Panamá, and onto the Pacific slopes of the Andes of Colombia and northwestern Ecuador (Trueb, 1974). Considering the results from Castroviejo-Fisher (2015), it is possible that one of the putatively distinct species they found within "*H. fasciatus*" could be *H. panamensis.* The type-locality for *H. panamensis* is located from Santa Isabel, Provincia Colón, Panamá (Stejneger, 1917). Santa Isabel, Provincia Colón, Panamá is east of the Panamá Canal. Our comparisons at this time, however, focused more broadly on samples from either from east and west of the Panama Canal.

Prior to the construction of the canal, the Isthmus of Panama was naturally lowland area, a thus also a natural barrier between the allopatric populations of *H. fasciatus.* Based on our review of morphological variation and in light of the genetic results from Castroviejo-Fisher et al. (2015) and the geographic separation we propose there is a new species of *Hemiphractus* in Panama currently confused with *H. fasciatus.*

CHAPTER 2

MATERIALS & METHODS

Specimens from the AMNH, KU, UMMZ, CNHM, UTACV and USNM collections were separated into batches representing the two focal upland regions of Panama; the few specimens available to us from South America were considered with the material from the Darien region, however the specimen form Ecuador (UMMZ 55523) was excluded from some comparisons because of poor condition and the sex could not be determined. Color descriptions are based on examinations of alcohol- preserved specimens. Standard body measurements were made to the nearest 0.1mm with digital calipers following Sheil and Mendelson (2001). Standard measurements to the nearest tenth of a millimeter of the skull and horns were replicated using methods illustrated by Trueb (1974) with two additional measurements: Horn Height and Horn Length (Fig. 1). Body measurements were log-transformed because our morphological measurements varied in scale over several orders of magnitude. A stepwise discriminate function analysis (DFA) was used to evaluate overall multivariate morphological distinctiveness of our a-priori determined allopatric populations. Analysis of Covariance (ANCOVA) tests were used to determine the univariate morphological distinctiveness of our focal populations. Morphological analyses and observations were conducted separately for males and females; small juveniles were excluded from the analyses.

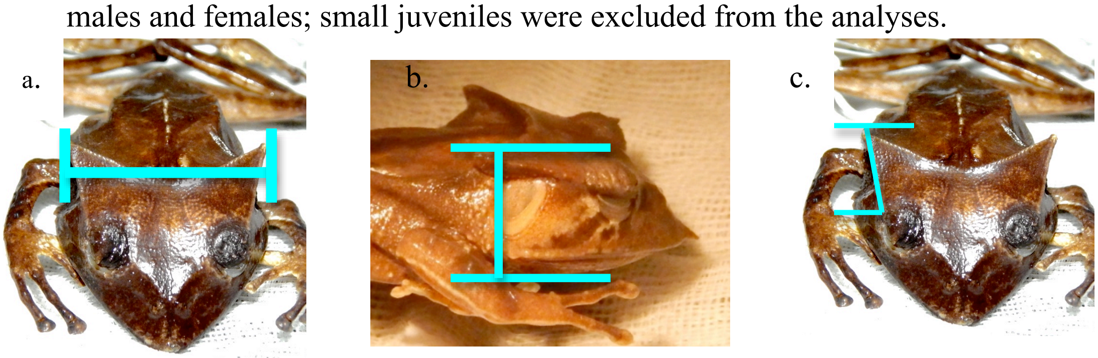

Figure 1. Horn Measurements. a. Horn width measurement. b. Horn height measurement c. Horn length. measurement.

CHAPTER 3

RESULTS

Standard Measurements

The DFA discovered two distinct centroids on the first two canonical axes (Figs 2, 3), and correctly classified all but two specimens The specimen misclassified for the females was numbered KU 93506 from Panamá, Darien SE slope Cerro Pirre 1440m. The specimen misclassified for the males was numbered KU 154992 from Panamá, Darien S slope Cerro Serrania de Pirre 1100 m. The stepwise analyses of the individual variables were not found to be different, however the multivariate centroids for both the males and females were not overlapping.

Results of the ANCOVA's on the individual variables for males were statistically significant for Width of the Horns (Fig 4. p= 0.0262), the Horn Height (Fig 5. p=0.0013). Horn Length (Fig 6. p=0.0503) was approaching traditional significance and with a larger sample number would likely be significant. Results of the ANCOVA's on the female specimens found a significant value only for Diameter of Orbit, however we are concerned about effect of the small sample size (n=15 females total; n=4 for the western samples). We are suspect of this particular statistical result and, there, it will not be considered further. Here we present only the ANCOVA results that were statistically significant.

Table 1. Discriminate Function Analysis for Females. All but one specimen was classified correctly. The misclassified specimen was KU 93506; Panamá, Darien SE slope Cerro Pirre, 1440m.

Actual	Predicted	
GROUP	**1**	**2**
1	4	0
2	1	10

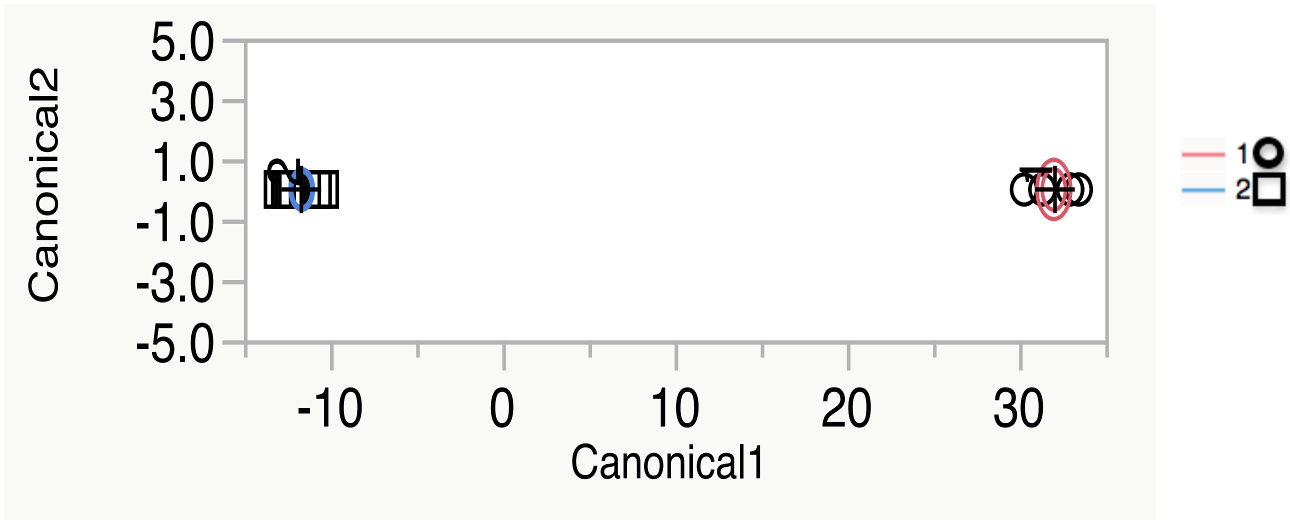

Figure 2. Results of Discriminate Function Analysis of Females. The canonical graph for the female specimens showing separation of the eastern (square symbols) and western (circle symbols) samples along Canonical Axis 1.

Table 2. Discriminate Function Analysis of Males. All but one specimen was classified correctly. The misclassified specimen was KU 154992, Panama, Darien S slope Cerro Serrania de Pirre 1100 m.

Actual	Predicted	
GROUP	1	2
1	11	0
2	1	10

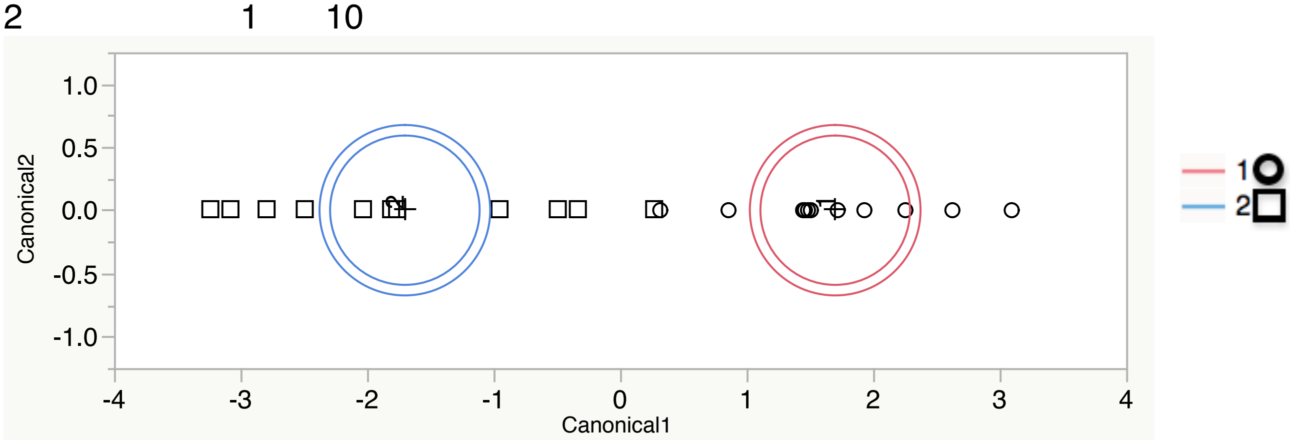

Figure 3. Results of Discriminate Function Analysis of Males. The canonical graph for the male specimens showing separation of the eastern (square symbols) and western (circle symbols) samples along Canonical Axis 1.

Table 3. Horn Width. Results of the Analysis of Covariance on males showing the effect of the two populations on Horn Width with SVL being the independent variable (P GROUP=0.0262, P SVL<0.0001).

Term	Estimate	Std Error	Prob>|t|
Intercept	-0.608278	0.173525	0.0024*
GROUP	0.0131582	0.005458	0.0262*
SVL	1.1547249	0.104226	<.0001*

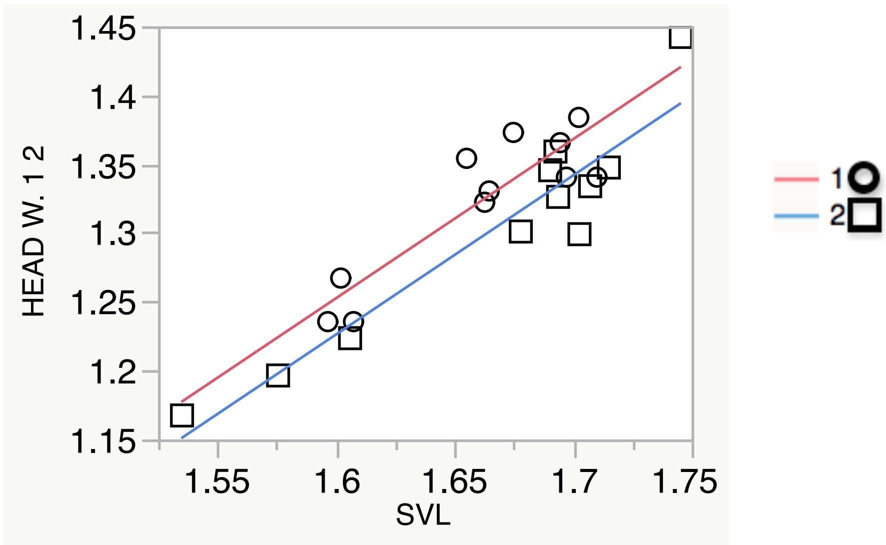

Figure 4. Horn Width ANCOVA plot showing the relationship between Horn Width and SVL (the independent variable) among males of the two populations in western (circles) and eastern (squares) of *H. fasciatus*.

Table 4. Horn Height. Results of the Analysis of Covariance on males showing the effect of the two populations on Horn Height with SVL being the independent variable (P GROUP=0.0013, P SVL<0.0001).

Term	Estimate	Std Error	Prob>|t|
Intercept	-0.576662	0.219581	0.0166*
GROUP	0.0261265	0.006907	0.0013*
SVL	0.9532196	0.13189	<.0001*

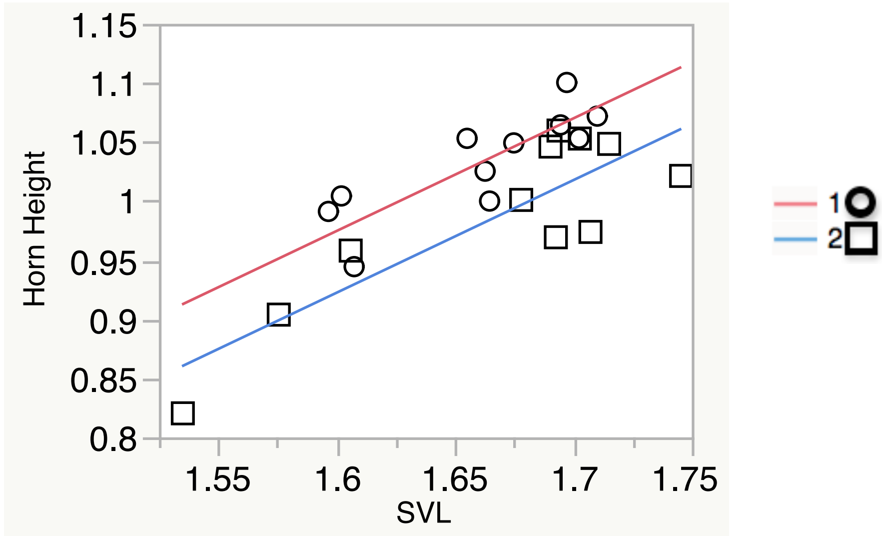

Figure 5. Horn Height. ANCOVA plot showing the relationship between Horn Height and SVL (the independent variable) among males of the two populations in western (circles) and eastern (squares) of *H. fasciatus*.

Table 5. Horn Length. Results of the Analysis of Covariance on males showing the effect of the two populations on Horn Length with SVL being the independent variable (P GROUP=0.0503, P SVL<0.0001).

Term	Estimate	Std Error	Prob>|t|
Intercept	-1.355304	0.188601	<.0001*
GROUP[1]	0.0123973	0.005933	0.0503
SVL	1.4109246	0.113282	<.0001*

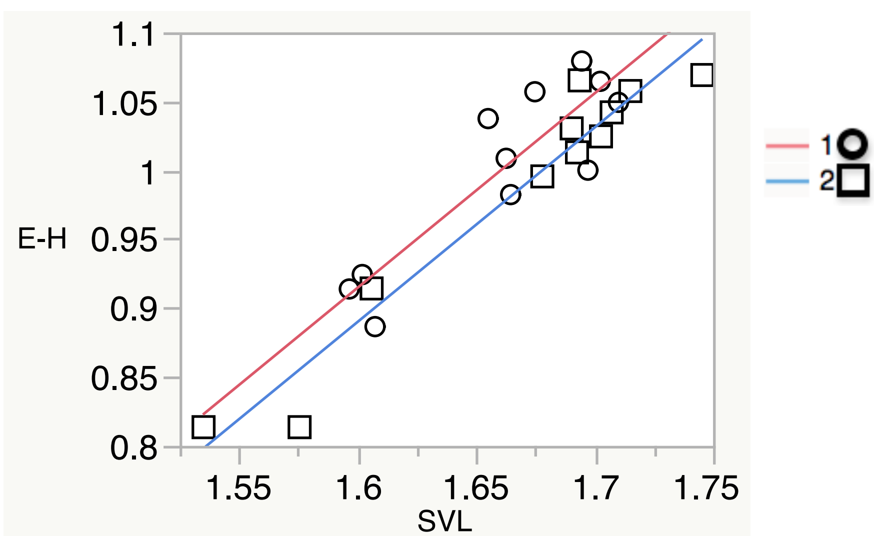

Figure 6. Horn Length. ANCOVA plot showing the relationship between Horn Length and SVL (the independent variable) among males of the two populations in western (circles) and eastern (squares) of *H. fasciatus*.

Table 6. Diameter of Orbit In Females. Results of the Analysis of Covariance on females showing the effect of the two populations on Diameter of Orbit with SVL being the independent variable (P GROUP=0.0183, P SVL<0.0001).

Term	Estimate	Std Error	Prob>ltl
Intercept	-0.610322	0.178659	0.0051*
GROUP[1]	0.0157646	0.005777	0.0183*
SVL	0.8161989	0.101023	<.0001*

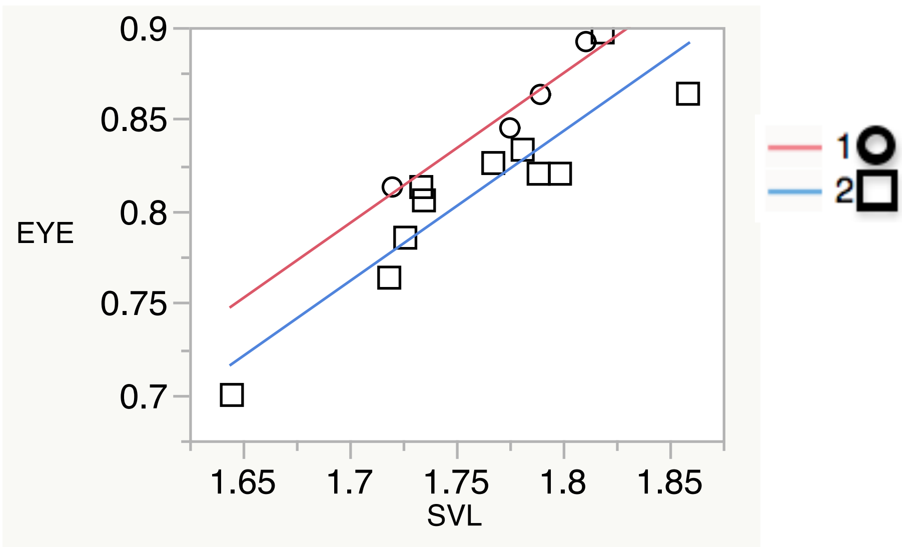

Figure 7. Diameter of Orbit In Females. ANCOVA plot showing the relationship between Diameter of Orbit and SVL (the independent variable) among females of the two populations in western (circles) and eastern (squares) of *H. fasciatus*.

Qualitative Characteristics

Two qualitative characteristics were found to differentiate the eastern and western populations. A distinct angular divot along the lateral margin of the horns is evident in the eastern specimens, whereas this margin of the horn is straight in the western specimens (Fig. 8). Additionally, we noted that the texture on the skin on the throat in the western specimens has large, uniformly spaced tubercles, while the eastern specimens have very small, randomly spaced tubercles (Fig. 9)

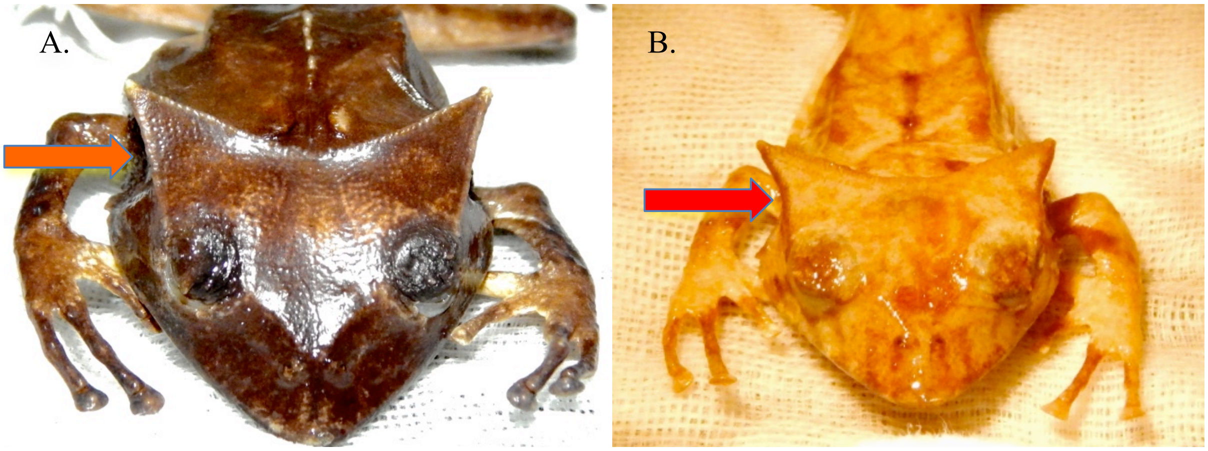

Figure 8. Horns Comparison of horns for the western group labeled a. and the eastern group labeled B. The arrow marks the divot in B. The lack thereof in A. is marked by an arrow as well.

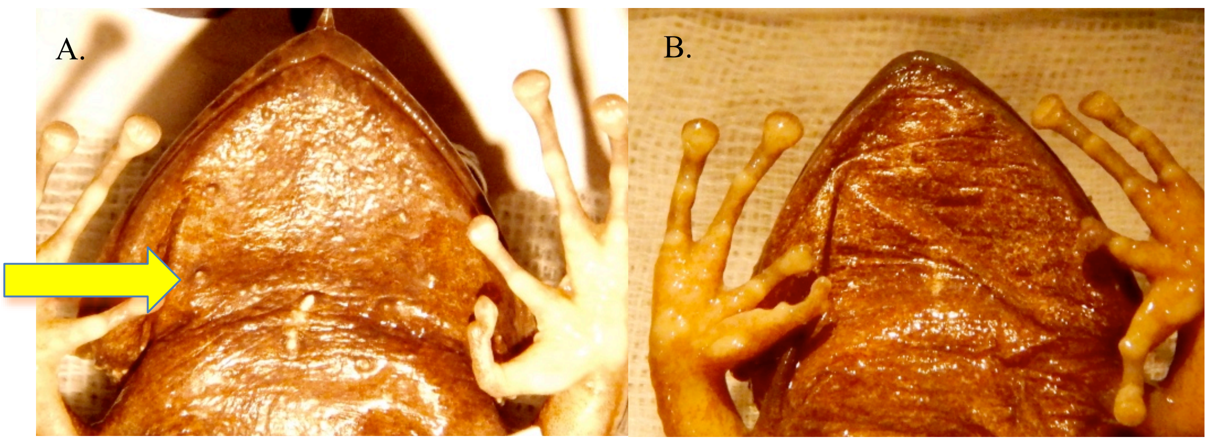

Figure 9. Throat Texture The texture of the throat of the western, A, versus the eastern group, B. The arrow points out the large uniform tubercles on A versus no visibly large tubercles on B.

CHAPTER 4

DISCUSSION

The sample size for both male and female were fairly low due to ongoing declines of frog species in Lower Central America (Lips et al., 2006), as well as the still-remote nature of some of the known localities for these frogs. I view the statistical analyses of the measurements were only reliable for the male specimens, given the low sample sizes available for females. Therefore, our morphological discrimination between the two populations is based on variation among male specimens alone. Notably, the differences we found were related to the horns: horn width, horn height, as well as a distinctive angular divot on the lateral margins. My statistical and qualitative assessments indicate that the eastern and western populations are morphologically distinct from another.

Considered together, with the genetic data from Castroviejo-Fisher et al. (2015), the allopatric geographic distribution of the specimens, and the morphological variation reported herein, we can conclude that there is a undescribed new species of *Hemiphractus* in western Panama that is currently classified as *H. fasciatus.* A new scientific name is needed as well as a holotype must be determined to classify this new species. Additional specimens, and data will be required to best assess referral of specimens from South America, as well as the status of *H. panamensis.*

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